

Applicant: Jay M. Short
U.S. Serial No.: 09/214,645
Filed: September 27, 1999
Page 5 of 11

Docket No.: DIV-1220-2

AMENDMENTS TO THE CLAIMS:

Complete Listing of Claims and Claim Status

16. (Previously added) A method for producing a mutagenized polynucleotide encoding a polypeptide having a desired property comprising:

blocking or interrupting a polynucleotide synthesis process by contacting a polynucleotide encoding a polypeptide or regulating expression of a polypeptide, with one or more agents that block or interrupt synthesis of the polynucleotide, wherein the agent is selected from UV light, one or more DNA adducts, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerase, cold or heat, chain terminators, polymerase inhibitors and poisons, and

subjecting said polynucleotides to denaturation, hybridization, elongation and selection to produce a mutagenized polynucleotide encoding a polypeptide having the desired property.

17. (Previously added) A method for producing a mutagenized polynucleotide encoding a polypeptide having a desired property, said method comprising:

(a) blocking or interrupting a polynucleotide synthesis process by contacting a polynucleotide encoding a polypeptide or regulating expression of a polypeptide, with at least one agent that blocks or interrupts synthesis of the polynucleotide, wherein the agent is selected from UV light, one or more DNA adducts, DNA intercalating agents, DNA binding proteins, triple helix forming agents, competing transcription polymerase, cold or heat, chain terminators,

Applicant: Jay M. Short
U.S. Serial No.: 09/214,645
Filed: September 27, 1999
Page 6 of 11

Docket No.: DIV-1220-2

polymerase inhibitors and poisons, to provide a plurality of single or double-stranded polynucleotides;

(b) denaturing the plurality of single or double-stranded polynucleotides to produce single-stranded polynucleotides;

(c) incubating the single-stranded polynucleotides with a polymerase under conditions which result in annealing of the single-stranded polynucleotides at regions of homology between the single-stranded polynucleotides and under conditions which promote synthesis of mutagenized polynucleotides, and;

(d) expressing at least one of the mutagenized polypeptides, wherein the polypeptide is identified as possessing a desired property, thereby producing a mutagenized polynucleotide encoding a polypeptide having a desired property.

18. (Previously added) The method of claim 16 or 17, wherein the DNA adduct is selected from the group of: UV light; (+)-CC-1065; (+)-CC-1065-(N3-Adenine); a N-acetylated or deacetylated 4'-fluoro-4-aminobiphenyl adduct capable of inhibiting DNA synthesis; trivalent chromium; a trivalent chromium salt; a polycyclic aromatic hydrocarbon DNA adduct capable of inhibiting DNA replication; 7-bromomethyl-benz[α]anthracene; tris(2,3-dibromopropyl)phosphate; 1,2-dibromo-3-chloropropane; 2-bromoacrolein (2BA); benzo[α]pyrene-7,8-dihydrodiol-9-10-epoxide; a platinum(II) halogen salt; N-hydroxy-2-amino-3-methylimidazo[4,5-f]-quinoline; N-hydroxy-2-amino-1-methyl-6-phenylimidazo[4,5-f]-pyridine, a DNA intercalating agent, a DNA binding protein, a triple helix forming agent, a

Applicant: Jay M. Short
U.S. Serial No.: 09/214,645
Filed: September 27, 1999
Page 7 of 11

Docket No.: DIV-1220-2

competing transcription polymerase, a chain terminator, a polymerase inhibitor, a polymerase poison and any combination thereof.

19. (Currently amended) The method of ~~any of claims 16-18~~ claim 16 or claim 17, further comprising heating the polynucleotides and removing the DNA adduct or adducts from the polynucleotide.

20. (Previously added) A method for expressing a mutant polypeptide comprising producing a mutagenized polynucleotide by the method of claim 16 or 17, and expressing the polynucleotide encoded by the mutagenized polynucleotide.

21. (Previously added) A mutagenized polypeptide, having a predefined desired activity, encoded by the mutagenized polynucleotide produced by the method of claim 16 or 17.

22. (Previously added) A vector comprising a mutagenized polynucleotide produced by the method of claim 16 or 17.

23. (Previously added) A polypeptide comprising at least one sequence segment expressed from a mutagenized polynucleotide produced by the method of claim 16 or 17.

24. (Previously added) The method of claim 16 or 17, further comprising amplifying one or more of the recombinant polynucleotides.

25. (Previously added) The method of claim 17, wherein steps (b) and (c) are repeated one or more times.

Applicant: Jay M. Short
U.S. Serial No.: 09/214,645
Filed: September 27, 1999
Page 8 of 11

Docket No.: DIV-1220-2

26. (Previously added) The method of claim 17, further comprising releasing and/or removing the DNA adduct prior to step (b).
27. (Previously added) The method of claim 17, wherein the DNA adduct is released and/or removed by heating a solution comprising the polynucleotides prior to step (b).
28. (Previously added) The method of claim 16 or 17, wherein the desired property comprises a phenotypic characteristic of the mutagenized polynucleotide or the polypeptide encoded thereby.
29. (Previously added) The method of claim 28, wherein the phenotypic characteristic comprises promotion of transcription of linked polynucleotide, or binding to a protein.
30. (Previously added) The method of claim 16 or 17, wherein the desired property comprises a functional characteristic.
31. (Previously added) The method of claim 30, wherein the functional characteristic comprises binding to a predetermined biological macromolecule.
32. (Previously added) The method of claim 30, wherein the functional characteristic comprises receptor binding.
33. (Previously added) The method of claim 16, wherein selection comprises screening a library of polynucleotides.
34. (Previously added) The method of claim 16 or 17, wherein the polynucleotide encodes an antibody, a protein receptor, a peptide oligosaccharide, or a virion.

Applicant: Jay M. Short
U.S. Serial No.: 09/214,645
Filed: September 27, 1999
Page 9 of 11

Docket No.: DIV-1220-2

35. (Previously added) The method of claim 16 or 17, wherein the blocking or interrupting is *in vitro*.
36. (Previously added) The method of claim 16, wherein the polynucleotides are subjected to amplification or selection *in vivo*.
37. (Previously added) The method of claim 17, wherein any one or more of steps (b), (c), and (d) or any combination thereof are *in vivo*.
38. (Previously added) The method of claim 16 or 17, wherein the polynucleotide encodes interleukin I, tPA, a ribozyme, a hormone, a receptor or an intracellular protein.
39. (New) The method of claim 18, further comprising heating the polynucleotides and removing the DNA adduct or adducts from the polynucleotide.